Antimicrobial activities of essential oil from *Artemisiae argyi* leaves

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Abstract: A study was conducted to determine the antimicrobial activities of essential oil from *Artemisiae argyi* leaves. The sample of the essential oil was analyzed by GC–MS. From 18 compounds representing the oils, Eucalyptole (18.42%), Spathulenol (14.32), 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (3.10%), 3-Carene (2.64%) appeared as the main components. The screening of antimicrobial activity of the essential oil was evaluated using agar diffusion and broth microdilution methods. Gram-positive bacterial were more sensitive than gram-negative bacterial of the 8 microorganisms, and *Staphylococcus aureus* ATCC 6538 showed the lowest MIC (0.3125%) and MBC (0.625%). In the disc diffusion assay, *Staphylococcus epidermidis* ATCC 49134 and *Bacillus subtilis* ATCC 6633 showed obvious inhibitory activity. Survival curve showed that, 2MIC of *Artemisiae argyi* essential oil had a lethal effect on *Candida albicans* within the first 1 h. Results presented here suggest that the essential oil of *Artemisiae argyi* leaves possesses antimicrobial properties, and provides scientific foundations for exploition of *Artemisiae argyi*.

Keywords: *Artemisiae argyi* leaves; Essential oil; Antimicrobial activity
CLC number: R284 **Document code**: A **Article ID**: 1007-662X(2006)04-332-03

Introduction

Artemisia species, widespread throughout the world, are important medicinal plants, which are receiving phytochemical attention due to the biological and chemical diversities (Tan *et al*. 1998). *Artemisias* (Compositae) are one of the most popular plants in Chinese traditional preparations and frequently used for the treatment of diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses.

Recently, because of the possible toxicities of the synthetic antimicrobial, increasing attention has been directed toward the natural resources (Naimiki,1990), natural products, including essential oils and various extracts of plants have provoked interest as sources of for the treatment of many infectious diseases. Particularly, the antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds 1996; Lis-Balchin 1997).

In this study, the antimicrobial capacities of the essential oil from *Artemisiae argyi* leaves were investigated. The antimicrobial activities were determined by using agar well-diffusion, agar disc diffusion and broth microdilution methods.

Materials and methods

Material and microorganisms

The essential oil was supplied by Xiamen Peony Perfume &Chemical Industry CO., LTD. The antimicrobial activity of the essential oil from *Artemisiae argyi* leaves was evaluated using a panel which included laboratory control strains obtained from the Institute of Applied Microbiology, Heilongjiang Academy of Sciences (Harbin, Hei longjiang, China): gram-positive bacteria: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 49134, *Candida albicans*, *Aspergillus niger V. Tiegh*; gram-negative bacteria: *Escherichia coli* ATCC 11229, *Proteus vulgaris*, *Pseudomonas aeruginosa*.

Methods

Essential oil and Gas Chromatography-Mass Spectrometry analysis

The composition of the essential oil has been reported before (Yin *et a*, 1999). And our analysis of the essential oil was performed using a VG platform Ⅱ GC-MS system equipped with a DB-5MS capillary column (30×0.25 mm i.d.; film thickness 0.25 μm). For GC–MS detection, an electron ionisation system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. The temperatures of injector and detector MS transfer line were set at 160 ºC and 220 ºC, respectively. Column temperature was initially kept at 60 ºC for 2 min, then gradually increased to 100 ºC at a rate of 3 ºC·min^{-1}, held for 4 min and finally raised to 220 ºC at 6 ºC·min^{-1},. 0.1μL essential oil were injected manually. The components were identified by comparison of their relative retention times and mass spectra with those of standards (for the main components), NIST library data of the GC–MS system and literature data (Adams 2001).

Microbial Cultures

Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 1.0×10^5 CFU/mL for bacteria and for fungal strains. 96-well microtiter plate injected with fungal strains was incubated at 28 ºC for 72h, and the bacteria were incubated at 37 ºC for 24 h.

Antimicrobial assay

Two different methods were employed for the determination of *in vitro* antimicrobial activities of the essential oil of *Artemisiae argyi* leaves: an agar disc diffusion method and broth microdilution method. The minimum inhibitory concentration (MIC), minimum bactericidal (MBC) (Hernandez *et al*. 2004) of the essential oil against the test microorganisms were determined

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Received date: 2006-09-10;
Accepted date: 2006-10-20
Responsible editor: Chai Ruihai
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by the broth microdilution method. The MIC, MBC, the inhibition zone and time-kill curve of Streptomycin were also determined in parallel experiments in order to control the sensitivity of the test microorganisms.

Disc Diffusion Method

A suspension of the tested microorganism (0.1 mL of 10^5cells·mL^{-2}) was spread on the solid media plates. Nutrient agar and Czapek Dox Agar sterilized in a flask and cooled to 45–50 ºC were distributed to sterilized Petri dishes with a diameter of 9 cm (15 mL). The filter paper discs (6 mm in diameter) were individually impregnated with 5µl of the *Artemisiae argyi* leaves essential oil and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37 ºC for 24 h and at 28 ºC for 72 h for the fungal strains. The diameters of the inhibition zones were measured in millimetres.

Broth microdilution method

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) according to the National Committee for Clinical Laboratory Standards (NCCLS, 1999). The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The MBC is defined as the lowest concentration of the essential oil at which inoculated microorganisms were completely killed. To determine MBC, 10µL broth was taken from each well and inoculated in Nutrient for 24 h at 37 ºC for bacteria or in Czapek Dox Agar for 72 h at 28 ºC for the fungi. All determinations were performed in duplicate and two growth controls consisting of Nutrient Agar and Czapek Dox agar medium was included. The *Artemisiae argyi* leaves essential oil served as a positive control. All tests were performed in Nutrient broth and Czapek-Dox broth supplemented with ethanol at final concentration of 0.5% (v/v) for all microorganisms. Serial doubling dilutions of the oils were prepared in a 96-well microtiter plate ranged from 5.00% to 0.039%.

Survival curve

The bactericidal kinetic assay was performed by using appropriate concentrations of essential oil, that was control, 1/2 MIC, MIC(MBC) and 2MIC, according to the method described by Avila *et al*. (Avila, *et al*. 1999)

Results and conclusion

Gas chromatography-mass spectrometry analysis

The sample of the essential oil was analyzed by GC–MS. From 18 compounds representing the oils, Eucalyptole (18.42%), Spathulenol (14.32%), 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (3.10%), 3-Carene (2.64%) appear as the main components.

Inhibition zone of essential oil from *Artemisiae argyi* leaves

The diameter of the inhibition zone is expressed in millimeter including the disc (6 mm). Table 1 lists the inhibitory zone(mm) values for the microorgnisms studied. From it we can see it clearly that, of the 8 test microorganisms studied in the disc diffusion assay, *Staphylococcus epidermidis* ATCC 49134 and *Bacillus subtilis* ATCC 6633 showed obvious inhibitory activity, and 1 (v/v) essential oil and 1/2 (v/v) essential oil have high activity to all microorganisms except *Pseudomonas aeruginosa.*

Table 1. Results of the inhibition zone of the essential oil from *Artemisiae argyi* leaves

Microorganisms	Diameter(mm) The concentration of the oil (v/v)					The diameter of Streptomycin (1mg/ml)
	1	0.5	0.25	0.125	0.0625	
Staphylococcus epidermidis ATCC 49134	18	14	10	7	6	17
Staphylococcus aureus ATCC 6538	16	12	9	7	6	20
Proteus vulgaris	16	14	7	6	6	25
Bacillus subtilis ATCC 6633	18	11	8	6	6	17.5
Escherichia coli ATCC 11229	15	12	7	6	6	12
Pseudomonas aeruginosa	7	6	6	6	6	15
Candida albicans	13	8	6	6	6	16
Aspergillus niger V. Tiegh	12	8	7	6	6	6

MIC and MBC of the essential oil from *Artemisiae argyi* leaves

Results of the MIC and MBC study are showed in Table 2. Gram-positive bacteria were more sensitive to essential oil from *Artemisiae argyi* leaves than gram-negative bacteria. *Staphylococcus aureus* ATCC 6538 presents the lowest MIC (0.3125%) and MBC (0.625%) values. Additionally, confirmed by both MICs and MBCs data, the oil exhibited significant antimicrobial activity at low concentration against microorganisms tested.

Survival curve of essential oil of *Artemisiae argyi* leaves

Fig. 1 shows the effect of the essential oil on a gram-positive bacterium (*Candida albicans*). 1/2MIC only inhibit microorganisms within the first 8h, and MIC(MBC) had a lethal effect on bacteria within the first 4 h, while 2MIC had a lethal effect on bacteria within the first 1 h.

Table 2. Results of the MIC and MBC of the *Artemisiae argyi* leaves essential oil

Bacterial strain	MIC (%)	MBC (%)
Staphylococcus epidermidis ATCC 49134	1.25	2.5
Staphylococcus aureus ATCC 6538	0.3125	0.625
Proteus vulgaris	0.625	1. 25
*Bacillus subtilis*ATCC ATCC 6633	0.625	>5
Escherichia coli ATCC 11229	0.625	0. 625
Pseudomonas aeruginosa	1.25	1.25
Candida albicans	0.3125	0.3125
Aspergillus niger V. Tiegh	0.625	2.5

The essential oil of *Artemisiae argyi* leaves is constituted mainly by monoterpenes and sesquiterpenes. The major components are: Eucalyptole (18.42%), Spathulenol (14.32), 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (3.10%), 3-Carene (2.64%).

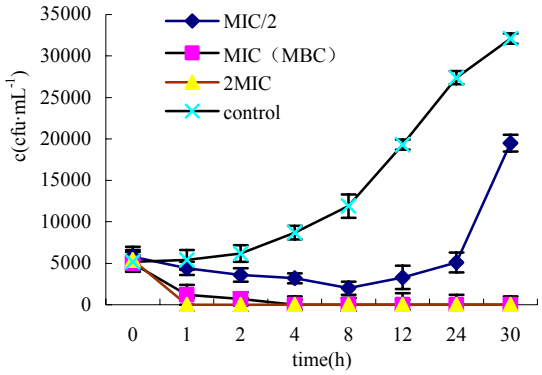

Fig. 1 Survival curve of *Candida albicans* exposed to essential oil of *Artemisiae argyi* leaves.

The essential oil was added to each experimental culture in zero time. The concentrations used for *Candida albicans* were: 0.625% (1/2 MIC), 0.3125% (MIC, MBC) and 0.15625% (2MIC), the control tube did not contain essential oil.

The two different screening methods examined was in accordance, normally, larger inhibition zone correlated with lower MICs. The essential oil of *Artemisiae argyi* leaves presented antibacterial activity against the tested strains, showing the biggest inhibition zones in the two strains of *Staphylococcus epidermidis* ATCC 49134 and *Bacillus subtilis* ATCC 6633 (Table 1). *Candida albicans* presented the lowest MIC and MBC values (Table 2). Minimum inhibitory concentrations (MIC, MBC) had a bacteriostatic effect on bacteria within the first 4 h, while the 2MIC had a lethal effect on bacteria within the first 1 h. The present study shows that the essential oil from *Artemisiae argyi* leaves has a good prospective for further exploitation.

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